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Manganese toxicity thresholds for restoration grass species

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Mn phytotoxicity thresholds for restoration grasses should be useful for risk assessments of metal-contaminated lands.

Abstract

Manganese toxicity thresholds for restoration plants have not been established. As a result, ecological risk assessments rely on toxicity thresholds for agronomic species, which may differ from those of restoration species. Our objective was to provide Mn toxicity thresholds for grasses commonly used in restoration. We used a greenhouse screening study where seedlings of redtop, slender wheatgrass, tufted hairgrass, big bluegrass, basin wildrye, and common wheat were grown in sand culture and exposed to increasing concentrations of Mn. The LC50, EC50-plant, EC50-shoot, EC50-root, PT50-shoot, and the PT50-root were then determined. Phytotoxicity thresholds and effective concentrations for the restoration species were generally higher than values reported for agronomic species. Our estimates of PT50-shoot for the five restoration grasses range from 41,528 to 120,082 mg Mn kg^{-1}. Measures of EC50-plant for these restoration grasses ranged from 877 to >6,000 mg Mn l^{-1}. These thresholds might be more useful for risk assessors than those based on crop plants that are widely used.

Keywords: Phytotoxicity; Manganese pollution; Restoration; Risk assessment

1. Introduction

Manganese is a common metal in the Earth's crust and its presence in soils mainly results from Mn in the parent material. It is also an essential micronutrient for plants. Mn plays a major role as an enzyme activator and it is an essential constituent of the manganese-containing superoxide dismutase that protects tissues from the toxic oxygen free radicals released in various enzyme reactions (Marschner, 1995).

Human practices have raised Mn content and availability in many soils. Mine tailings and metal smelters increase soil Mn concentration and availability with subsequent effects on vegetation structure and composition (Zheljazkov and Nielsen, 1996; Wong et al., 1983). Long-term and heavy dose applications of sewage-sludge (biosolids) or other organic amendments to agricultural soils and soil anaerobic conditions such as waterlogging or poor drainage may also lead to an increase in the content or availability of Mn and other heavy metals (Ramachandran and D'Souza, 1997). The availability of Mn in soils is largely controlled by soil pH (Smith and Paterson, 1990). However, the pH of the rhizosphere is more crucial for determining Mn availability to plants (Reisenaur, 1988).

Little is known about metal toxicity thresholds in perennial rangeland species. Increasing knowledge about the sensitivity to heavy metals of wild, non-agricultural plant species would help land managers make appropriate decisions when planning the restoration of Mn contaminated soils. Much of the research dealing with Mn-phytotoxicity thresholds in plants has

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been carried out with agricultural species. There is little information about the effects of high Mn levels on those non-agricultural plant species suitable for restoring and revegetating Mn contaminated soils (Prodgers and Inskeep, 1991).

Manganese uptake by plants mainly occurs in the reduced-bivalent form, thus its availability increases in acidic soils or anaerobic conditions. High Mn levels in soil may lead to plant nutrient imbalances, especially in relation to other divalent cations such as Mg^{2+} and Ca^{2+} (Marschner, 1995; Cenni et al., 1998) and metals such as Zn (de Varennes et al., 2001). In general, nutrient uptake, especially in relation to elements entering the roots by diffusion, may be hampered by Mn due to Mn inhibition of root hair production and reduction of stomata dimensions (Lidon, 2002). High substrate Mn may thus reduce plant growth due to other nutrient deficiencies instead of Mn toxicity (Langheinrich et al., 1992). In fact, one of the first symptoms associated with Mn toxicity is related to both Ca and Mg deficiencies (Marschner, 1995).

Two plant strategies have been proposed for tolerating high substrate Mn concentrations. One mechanism is related to the internal tissue tolerance by which high Mn concentrations are permitted within the leaves or roots with no apparent toxic effect (Horst, 1988; Ross and Kaye, 1994). The second strategy is to reduce Mn uptake and therefore avoid high tissue Mn concentration (Quartin et al., 2001). Whole-plant or specific tissue Mn concentration generally increases with increasing substrate concentration of Mn, but different effects are often observed for different species, varieties or genotypes in relation to their sensitivity to Mn (Scott et al., 1998; Lee et al., 1996; Choi et al., 1996). Mahmoud and Grime (1977) observed that the susceptibility of a given species to high Mn levels was related to their ecology and the ability of the species to tolerate acidic soils.

In establishing metal toxicity thresholds for plants it is important to consider several characteristics of toxicity: the quantity and species of metal, the route of exposure, the distribution of the metal both spatially and temporally, the type and severity of injury, and the time needed to produce the injury (Ross and Kaye, 1994). Several methods for describing metal toxicity in plants have been proposed. Most of these have been derived from measures of human or animal health assessments. A discussion of these methods is presented in Ross and Kaye (1994). The lethal concentration (LC) is the concentration of a toxin that kills a specified percentage of organisms. Effective concentration (EC) is the concentration of a toxin that produces an observable negative effect in the organism. The phytotoxicity threshold (PT) is the tissue concentration of a plant that corresponds with a defined growth reduction.

Metal toxicity thresholds for plants can be used to estimate a plant's ability to establish and survive on a contaminated site. Unfortunately, there is a paucity of data on toxicity thresholds for native plant species (Ross and Kaye, 1994) and ironically, there is a lack of information for species that are used to restore heavy metal contaminated sites. Miles and Parker (1979) have identified Cd toxicity thresholds for seven plant species native to northwestern Indiana, and in previous work we have determined Zn (Paschke et al., 2000) and Cu (Paschke and Redente, 2002) toxicity thresholds for a variety of grass species. Others have attempted to establish toxicity thresholds for individual native plant species using a few metals (e.g. Pedersen et al., 2000; Symeonidis et al., 1985; Hogan and Rauser, 1979; Ehinger and Parker, 1979). Most work on metal effects on native plant species has focused on relative toxicity of species or ecotypes for selection and use in phytoremediation efforts (e.g. Ebbs and Kochian, 1997; Wu and Kruckeberg, 1985; Humphreys and Nicholls, 1984; Pollard, 1980). The vast majority of plant metal toxicity thresholds have been determined for agricultural species (reviewed by Gough et al., 1979).

Due to the paucity of Mn toxicity thresholds established for restoration species, ecological risk assessments and natural resource damage assessments conducted in the United States under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 must rely on toxicity thresholds established for agronomic species. These crop plants may have very different physiological characteristics and sensitivity levels than species used in the restoration of sites contaminated with metals and may therefore be inappropriate for these ecological assessments.

Many metal toxicity thresholds for plants are determined in greenhouse or laboratory experiments by growing plants in nutrient solutions containing known concentrations of metals (reviewed by Macnicol and Beckett, 1985). While these conditions do not mimic field conditions, they may provide a conservative first estimate of toxicity thresholds. Many factors that are lacking in solution culture experiments would be expected to alleviate metal toxicity stress to plants growing in the field. These factors include rhizosphere organisms such as mycorrhizae (Brown and Wilkins, 1985; Bradley et al., 1982; Jones and Hutchinson, 1986; Martino et al., 2000; Van Tichelen et al., 2001) and metal binding with soil organic matter (Alloway, 1990; Stevenson and Ardakani, 1972; Ghosh and Banerjee, 1997) and clays (Alloway, 1990). Other factors present in the field such as herbivory, competition and pathogens would act synergistically with metals to reduce a plant's ability to tolerate high metal concentrations. Thus, toxicity thresholds determined from solution culture experiments would likely be lower than actual field toxicity thresholds. However, given the extreme heterogeneity associated with soil organisms, organic matter, herbivory, competition and pathogens,

both within and between sites, it can easily be argued that metal toxicity data derived from solution culture experiments would have broader utility.

In previous studies (Paschke and Redente, 2002; Paschke et al., 2000), we have determined zinc and copper toxicity thresholds for several grass species that are commonly used in restoration activities in Western North America. In this paper, we describe a similar study of Mn toxicity thresholds for grass species used in restoration efforts. The objective of this study was to provide a better estimate of Mn toxicity thresholds for five grass species that are commonly used in restoration efforts in the Western United States. Until now, this information has been unavailable and, as a result, ecological risk assessments have relied on Mn toxicity thresholds established for agronomic species.

2. Materials and methods

2.1. Plant growth conditions

A greenhouse screening study was used to determine Mn toxicity thresholds for redtop (*Agrostis gigantea* Roth.), slender wheatgrass (*Elymus trachycaulus* [Link] Gould ex Shinners var. Pryor), tufted hairgrass (*Deschampsia caespitosa* (L.) Beauv.), big bluegrass (*Poa ampla* J. Presl var. Sherman), basin wildrye (*Leymus cinereus* [Scribn. & Merr.] A. Löve var. Magnar), and common wheat (*Triticum aestivum* L. var. Oslo). Common wheat is an agricultural crop and redtop was introduced to North America from Europe; the remaining species are native to the western US where they are commonly used in restoration and reclamation projects. Wheat seed was obtained from a local agricultural seed supplier and the restoration species were obtained from Granite Seed Company (Lehi, UT, USA), a company that typically supplies the restoration industry. Although previous studies have noted ecotypic metal tolerance variation in native plant species (Symeonidis et al., 1985; Hogan and Rauser, 1979; Ehinger and Parker, 1979), we used seed that would typically be used in the restoration of metal-contaminated sites as an approximation of species toxicity thresholds.

A sand culture technique was used to establish toxicity thresholds because many of these arid and semiarid grass species do not grow well in aerated solution culture. Approximately three seeds of each species were sown directly into 3.8- × 21-cm plastic Cone-tainer™ tubes (Stuewe & Sons, Corvallis, OR, USA). Each tube was filled with approximately 350 cm^3 of washed quartz sand (Quikrete® Play Sand) and the sand was covered with approximately 1 cm of perlite to retain moisture at the sand surface. Sand-filled tubes were rinsed daily with approximately 300 ml of water for 1 week prior to seed sowing. Preliminary tests showed the sand to have a pH of 6.93 (0.01 M $CaCl_3$). Although the pH of the media can be important for Mn availability in bulk soil, it has been demonstrated that the pH of the rhizosphere, which can be much lower than the pH of bulk soil, is the more important measure for determining plant uptake of Mn in greenhouse and field soils (Reisenaur, 1988). Tests of leachate from the sand-filled tubes found no detectable water soluble metals in this media. The pH of water, treatment solutions and plant nutrient solutions were not significantly altered by passage through growth containers filled with sand. A glass wool plug was put in the bottom of each container to keep the soil from escaping through drainage holes. After emergence, seedlings were thinned to one individual per tube.

Manganese treatment began when the seedlings were approximately 4 weeks old. All plants were provided with a complete nutrient solution (Miracle-Gro™ Nutriblend 21-18-18) on alternate days prior to Mn treatments. The fertilizer was applied at standard rate (50 ppm N) via a fertilizer injector. Forty-nine seedlings of each of the six species were exposed to one of seven supplemental Mn treatments: 0, 1000, 2000, 3000, 4000, 5000, or 6000 mg Mn l^{-1}. Manganese treatments were administered by application of $MnSO_4$ solutions on alternate days (MWF) with nutrient solution being added separately (TT). Plants were provided with water as needed on weekends (during the first 30 days of the experiment the small seedlings rarely required weekend watering). Nutrient solution, water and Mn treatments were applied in amounts that saturated the media as evidenced by drainage of solution out of the bottom of the tubes. This treatment regime was continued for 60 days. During the growth period, the greenhouse was maintained at 23 ± 8 °C, with an extended photoperiod of 16 h using 400 W Na vapor lamps that provided approximately 300 μmol m^{-2} s^{-1} of photosynthetically active radiation at a distance of 1.5 m.

2.2. Measures of toxicity

There are numerous measures of metal toxicity thresholds in plants (Ross and Kaye, 1994). In this study, we determined six commonly used measures of toxicity: The 60-day LC50 (the concentration of metal that kills 50% of the seedlings by 60 days), the 60-day EC50-plant (the concentration of metal that reduces seedling biomass by 50% after 60 days), the 60-day EC50-shoot (the concentration of metal that reduces shoot biomass by 50% after 60 days), the 60-day EC50-root (the concentration of metal that reduces root biomass by 50% after 60 days), the PT50-shoot (the shoot metal concentration corresponding to a 50% seedling biomass reduction), and the PT50-root (the root metal concentration corresponding to a 50%

seedling biomass reduction). The LC50 was determined from observations of plant status, alive or dead, at the conclusion of the greenhouse experiment. Sixty days after treatments began, seedlings were harvested and the sand was separated from the roots by gently washing under a stream of water. Roots were separated from shoots and both were dried to constant mass at 55 °C and weighed to determine EC50 values. Treatment effects on root, shoot and plant mass were also evaluated directly using univariate analyses. Differences between control and treatment means were tested using a Tukey's Studentized Range test ($\alpha = 0.05$) on SAS PROC GLM version 8.01 (SAS Institute, Inc., Cary, NC, USA). A subset of root and shoot samples (five plants from each species × Mn treatment combination) were then analyzed for Mn concentrations by HNO_3/$HClO_4$ digestion and analysis by inductively coupled plasma emission spectroscopy at the Soil and Plant Analysis Laboratory at Colorado State University.

Toxicity thresholds were calculated from the data by fitting them to linear and polynomial models using SAS version 8.01 (SAS Institute, Inc., Cary, NC, USA). The model (either linear or polynomial) that resulted in the best fit to the data, as determined by R^2 and p values, was used to calculate each toxicity threshold.

3. Results

Mortality varied greatly by species during the 60-day study period (Table 1). Redtop, basin wildrye and big bluegrass all had high survival with Mn treatment levels of 4000 mg l^{-1} or higher. Tufted hairgrass survived well up to 2000 mg l^{-1}, whereas slender wheatgrass and common wheat had very low survival even at the lowest treatment level of 1000 mg l^{-1}. These survival rates resulted in high estimates for LC50 (>4500 mg l^{-1}) for redtop, basin wildrye and big bluegrass (Table 2), a relatively moderate LC50 for tufted hairgrass (2568 mg l^{-1}) and a low LC50 for slender wheatgrass (248 mg l^{-1}). Survival of common wheat with the range of Mn concentrations that we tested was too low to calculate an LC50 for this taxa (<1000 mg l^{-1}). Trends in plant size for the various species exposed to Mn were similar to those of survival, with redtop and big bluegrass showing little reduction in plant size relative to other species at the higher treatment levels (Fig. 1). Individual big bluegrass plants did show reduced size (in both shoot and root mass) at treatment levels over 1000 mg l^{-1}. Whereas, in redtop only root biomass was reduced by Mn additions while shoot biomass increased relative to controls at treatment levels as high as 4000 mg l^{-1} resulting in few significant changes in overall plant size (root plus shoot) across the treatment gradient (Fig. 1A). No other species showed a significant increase in growth as a result of the Mn treatments (Fig. 1). It should be noted that the fertilizer solution that we provided to all plants twice a week, including controls, contained Mn (7 mg l^{-1}) intended to meet the plant's basic nutritional requirements.

Estimated EC50-shoot values ranged from 707 to greater than 6000 mg Mn l^{-1} (Table 2). Most of the restoration grass species had EC50-shoot values that exceeded the Mn concentration range tested (> 6000 mg l^{-1}). Common wheat (EC50-shoot <1000 mg l^{-1}) and slender wheatgrass (EC50-shoot 248 mg l^{-1}) appeared to be the most sensitive species with respect to Mn effects on shoot growth, with significant reductions in shoot mass occurring at 1000 mg Mn l^{-1} (Fig. 1). Roots of all of these grass species appeared to be more sensitive than shoots to Mn induced growth reductions (Fig. 1). Common wheat and slender wheatgrass were again the most sensitive species to Mn, with root growth being significantly reduced at 1000 mg l^{-1} (Fig. 1, Table 2). The effects of Mn on whole plant biomass were similar to those of roots and shoots, with common wheat and slender wheatgrass being sensitive and the majority of the restoration grasses showing less sensitivity to Mn.

Manganese was readily taken up in large amounts by all species (Fig. 2). Large amounts of Mn were retained in roots with generally lesser amounts translocated to shoots (Fig. 2). Calculated PT50-shoot values ranged

Table 1
Percent survival (after 60 days) of grass species exposed to various Mn treatment levels

Treatment	Species					
Mn (mg l^{-1})	Redtop	Slender Wheatgrass	Tufted Hairgrass	Basin Wildrye	Big Bluegrass	Wheat
0	100[a] (36)[b]	100 (49)	100 (48)	98 (49)	98 (48)	94 (49)
1000	89 (38)	18 (49)	100 (47)	96 (49)	100 (48)	2 (49)
2000	89 (37)	0 (49)	76 (46)	94 (49)	89 (44)	0 (49)
3000	86 (44)	0 (49)	9 (46)	73 (48)	60 (35)	0 (49)
4000	61 (44)	0 (49)	0 (49)	71 (48)	68 (22)	0 (49)
5000	53 (45)	0 (49)	0 (46)	53 (49)	44 (16)	0 (49)
6000	13 (43)	0 (48)	4 (45)	38 (47)	33 (48)	0 (49)

These survival values were used to estimate LC50 values (Table 2).

[a] Values are raw scores for survival of all of the seedlings in the experiment.

[b] The number of seedlings (n) used in each species by treatment combination. This number varied due to lack of germination in some of the tubes. The original number of tubes planted for each species by treatment combination was 49.

Table 2
Calculated manganese toxicity thresholds for lethal concentration (LC50), effective concentrations (EC50-shoot, EC50-root and EC50-plant), and phytotoxicity thresholds (PT50-shoot and PT50-root)

Species	Threshold type	R^2	p	Model	Calculated threshold (x)
Redtop	LC50	0.85	0.0033	$y=108.91-1.28^{-02}x$	4604
	EC50 shoot				>6000[a]
	EC50 root				>6000[a]
	EC50 plant				>6000[a]
	PT50 shoot	0.44	<0.0001	$y=142.10-7.67^{-04}x$	120082
	PT50 root				>48000[a]
Slender wheatgrass	LC50	0.49	0.0818	$y=52.99-1.20^{-02}x$	248
	EC50 shoot	0.32	<0.0001	$y=97.20-6.85^{-02}x+1.87^{-05}x^2-1.57^{-09}x^3$	886
	EC50 root	0.36	<0.0001	$y=96.95-6.90^{-02}x+1.86^{-05}x^2-1.54^{-09}x^3$	868
	EC50 plant	0.35	<0.0001	$y=97.07-6.88^{-02}x+1.86^{-05}x^2-1.56^{-09}x^3$	877
	PT50 shoot	0.75	0.0013	$y=148.66-1.55^{-03}x$	63446
	PT50 root				>29000[a]
Tufted hairgrass	LC50	0.81	0.0056	$y=101.62-2.01^{-02}x$	2568
	EC50 shoot				>6000[a]
	EC50 root				>6000[a]
	EC50 plant				>6000[a]
	PT50 shoot	0.61	0.0015	$y=123.87+8.98^{-05}x-2.88^{-08}x^2+2.85^{-13}x^3$	41528
	PT50 root	0.66	0.0005	$y=99.98+1.45^{-04}x+2.32^{-08}x^2+2.62^{-13}x^3$	36865
Basin wildrye	LC50	0.93	0.0004	$y=105.53-1.03^{-02}x$	5403
	EC50 shoot				>6000[a]
	EC50 root				>6000[a]
	EC50 plant				>6000[a]
	PT50 shoot	0.67	<0.0001	$y=137.06-2.59^{-03}x+1.00^{-07}x^2-1.14^{-12}x^3$	70944
	PT50 root	0.60	<0.0001	$y=112.02-4.09^{-04}x$	151630
Big bluegrass	LC50	0.92	0.0007	$y=105.27-1.17^{-02}x$	4736
	EC50 shoot				>6000[a]
	EC50 root				>6000[a]
	EC50 plant				>6000[a]
	PT50 shoot	0.61	<0.0001	$y=100.50-7.51^{-04}x$	67241
	PT50 root				>26900[a]
Wheat	LC50				<1000[a]
	EC50 shoot	0.55	<0.0001	$y=91.69-7.29^{-02}x+2.10^{-05}x^2-1.84^{-09}x^3$	707
	EC50 root	0.59	<0.0001	$y=90.58-7.77^{-02}x+2.28^{-05}x^2-2.01^{-09}x^3$	632
	EC50 plant	0.58	<0.0001	$y=91.11-7.54^{-02}x+2.20^{-05}x^2-1.93^{-09}x^3$	667
	PT50 shoot	1.00	<0.0001	$y=105.00-9.59^{-03}x$	5732
	PT50 root	0.96	0.0006	$y=100.21-7.98^{-03}x$	6295

Values for EC50s are mg Mn l^{-1}, values for PT50s are mg Mn kg^{-1}.
[a] Estimated threshold is outside the data range and can only be estimated based upon the highest or lowest treatment level for LC50 and EC50s or mean tissue concentration for PT50s.

from 5732 mg Mn kg^{-1} for common wheat to 120,082 mg Mn kg^{-1} for redtop. Estimated PT50-root values ranged from 6295 mg Mn kg^{-1} for common wheat to 151,630 mg Mn kg^{-1} for Basin wildrye.

4. Discussion

Metal toxicity thresholds in plants can be difficult to determine due to complex interactions between the toxic metal and other nutrient elements, as well as other complex biological and physical factors (Foy et al., 1978). Here, we have identified Mn phytotoxicity thresholds for several important restoration grass species and common wheat using a simplified approach that circumvents many of these experimental pitfalls.

Mean Mn concentrations for shoots in the control treatment, which received 7 mg Mn l^{-1} in fertilizer solution, were between 204 and 563 mg kg^{-1}. This range is well above the 10–30 mg kg^{-1} general plant Mn deficiency levels reported by Kabata-Pendias and Pendias (2001). This would seem to indicate that adequate Mn was provided to the plants in the fertilizer solution, but we did observe a significant increase in shoot growth of redtop at Mn applications of up to 4000 mg l^{-1} (Fig. 1A). This hormetic dose-response is typical

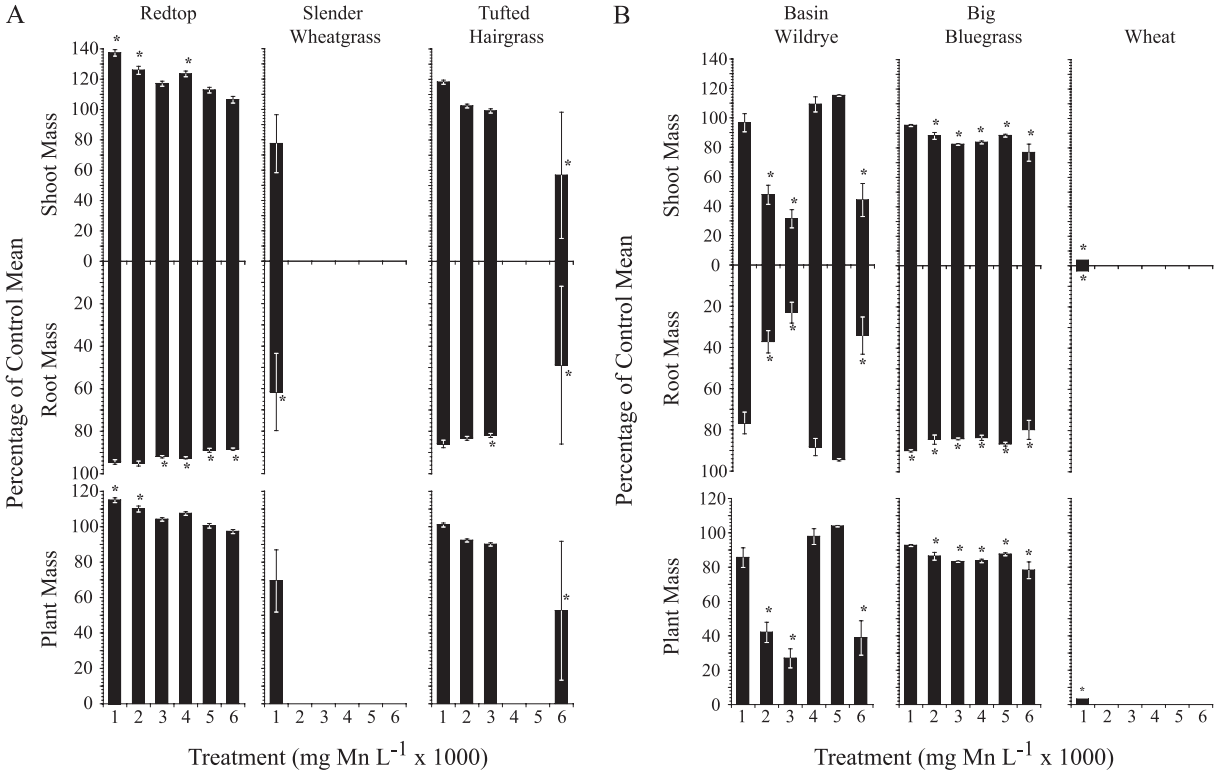

Fig. 1. Effect of Mn on plant biomass presented as a percentage of the control means. Panel A shows redtop, slender wheatgrass and tufted hairgrass. Panel B shows basin wildrye, big bluegrass and common wheat. Thin bars represent the standard error of the mean (n = between 49 and 1 depending on mortality of test plants during the study period). Treatment means that are significantly different from the corresponding control mean at $\alpha = 0.05$ by using Tukey's Studentized range test are indicated by an asterisk (*).

of many toxicological processes (Calabrese and Baldwin, 2003) but we were surprised to see it at such high Mn treatment levels for this species.

The phytotoxicity thresholds that we have determined for these reclamation species (Table 2) are generally higher than those values that have been reported for agronomic species (Table 3). The one agricultural species that we included in our study, common wheat, was the most sensitive species tested since it nearly always had the lowest values for each threshold type (Table 2). Foy et al. (1973) reported a Mn-EC50-shoot value of >32 mg l^{-1} and an EC50-root value of 16–32 mg l^{-1} for wheat. Our estimates of 707 and 632 for these same measures on common wheat are considerably higher. Other estimates of Mn-PT10-shoot for wheat are in the range of 200–2561 mg l^{-1} (Foy et al., 1973; Ohki, 1984; De Marco et al., 1995). These estimates for PT10 (the shoot metal concentration corresponding to a 10% seedling biomass reduction) are not directly comparable to our PT50-shoot estimate of 5732 mg l^{-1} for common wheat. However, our estimate appears to be reasonable for common wheat given our higher threshold criteria (50% biomass reduction versus 10%).

Few Mn phytotoxicity thresholds have been reported for nonagricultural grass species. Data from Jackson et al. (1995) can be used to estimate a Mn-PT50-shoot for buffalograss at $>14{,}100$ mg kg^{-1}. Data from Lee et al. (1996) indicate a similar Mn-PT50-shoot of $>14{,}900$ for Kentucky bluegrass. Our estimates of PT50-shoot for five nonagricultural grass species range from 41,528 mg Mn kg^{-1} for tufted hairgrass to 120,082 mg Mn kg^{-1} for redtop.

Roots appeared to be slightly more negatively affected by Mn than shoots (Fig. 1). This differential effect of Mn on roots versus shoots for various species indicates that a more robust measure of effective concentrations may be the EC50-plant. On sites with no existing vegetation, where PT measures are not possible, EC measures could be useful for selecting species and understanding site limitations in restoration planning where they can be related to levels of soil solution Mn. Monitoring soil solution Mn with lysimeters could accomplish this. Our measures of EC50-plant for restoration grasses ranged from 877 to more than 6000 mg Mn l^{-1}. These Mn phytotoxicity concentrations should be generally applicable to those obtained from lysimeter solutions. Under field conditions, Mn stress would act synergistically with other environmental factors (e.g. competition, disease, herbivory) and would result in greater mortality than was observed in this simple greenhouse study. We recognize that toxicity thresholds reported here are only approximations of

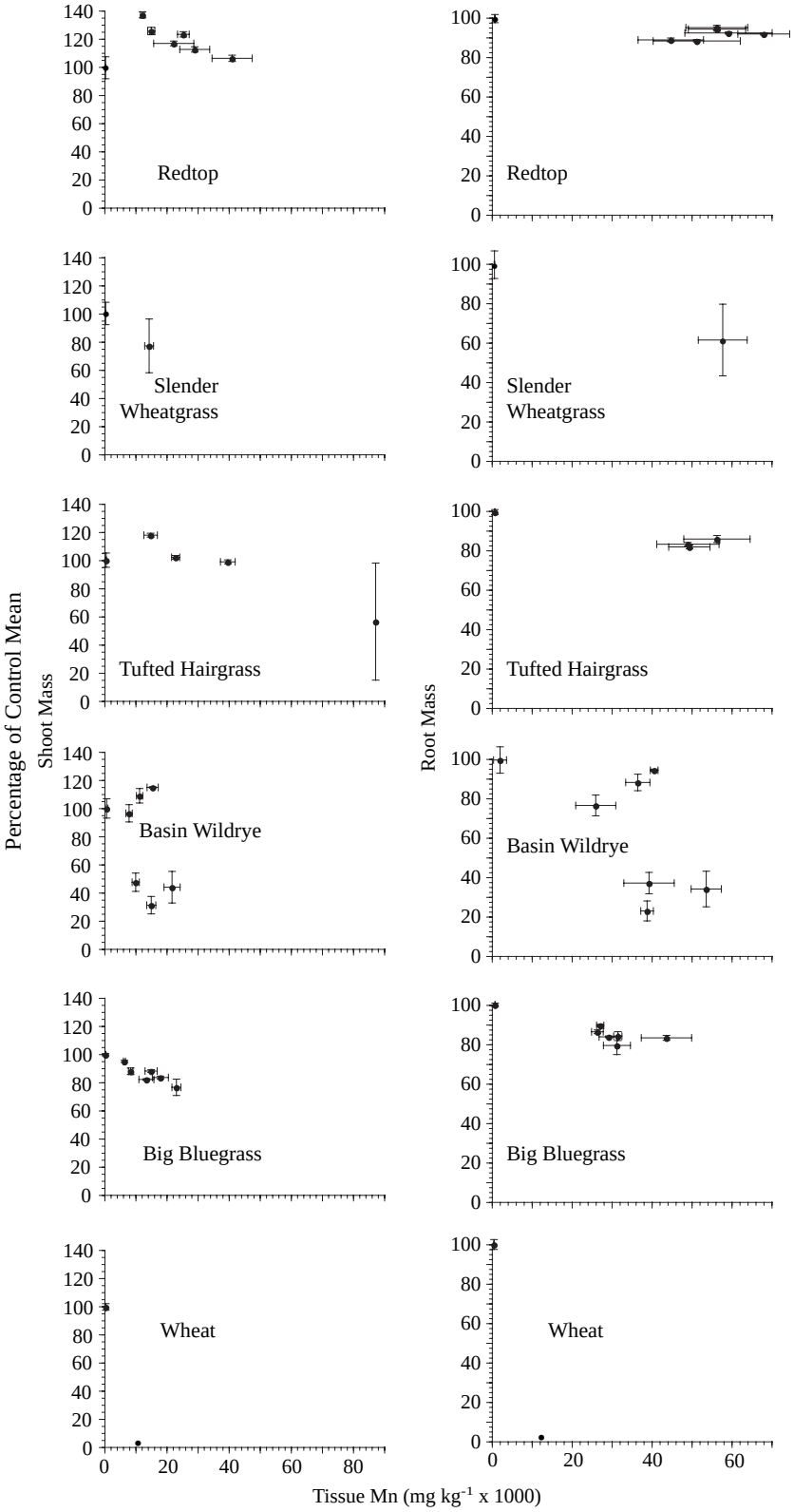

Fig. 2. Relationships between plant tissue Mn concentrations and growth reduction in shoots and roots of various grass species growing in sand culture and exposed to supplemental Mn treatments ranging from 0 (control) to 6000 mg Mn l^{-1}. Error bars represent the standard error of the mean. Note that axes for shoots and roots are not scaled uniformly.

Table 3
Mn phytotoxicity thresholds (PT) and effective concentrations (EC) for grasses that have been published, or have been calculated here from published data

Plant taxa	Threshold	Value[a]	Reference
Agricultural species			
Corn (*Zea mays* L.)	EC10-shoot	400	(Fageria, 2001)
	PT50-shoot	~4000	(Fageria, 2001)
Rice (*Oryza sativa* L.)	EC10-shoot	560	(Fageria, 2001)
	PT50-shoot	>5000	(Fageria, 2001)
Triticale (×*Triticosecale rimpaui* Wittm. [*Triticum aestivum*×*Secale cereale*])	EC50-shoot	>25	(Quartin et al., 2001)
	EC50-root	<25	(Quartin et al., 2001)
Wheat (*Triticum aestivum* L.)	PT10-shoot	396–2561	(Foy et al., 1973)
	EC50-shoot	>32	(Foy et al., 1973)
	EC50-root	16–32	(Foy et al., 1973)
	PT10-shoot	200–1100	(Ohki, 1984)
	PT10-shoot	373	(Ohki, 1985)
	PT10- shoot	570	(De Marco et al., 1995)
Nonagricultural species			
Buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm.)	EC50-shoot	>659	(Jackson et al., 1995)
	PT50-shoot	>14100	(Jackson et al., 1995)
Colonial bentgrass (*Agrostis capillaris* L.)	EC50-shoot	~100	(Mahmoud and Grime, 1977)
	EC50-root	~100	(Mahmoud and Grime, 1977)
Kentucky bluegrass (*Poa pratensis* L.) (cv. Touchdown)	EC50-shoot	>659	(Lee et al., 1996)
	PT50-shoot	>14900	(Lee et al., 1996)
Sheep fescue (*Festuca ovina* L.)	EC50-shoot	>200	(Mahmoud and Grime, 1977)
	EC50-root	>200	(Mahmoud and Grime, 1977)
Tall oatgrass (*Arrhenatherum elatius* (L.) Beauv. ex J. & K. Presl)	EC50-shoot	5–25	(Mahmoud and Grime, 1977)
	EC50-root	2-25	(Mahmoud and Grime, 1977)
Wavy hairgrass (*Deschampsia flexuosa* (L.) Trin.)	EC10-shoot	312	(Kroeze et al., 1989)
	EC10-root	>312	(Kroeze et al., 1989)
	EC50-shoot	>312	(Kroeze et al., 1989)
	EC50-root	>312	(Kroeze et al., 1989)
	EC50-shoot	>200	(Mahmoud and Grime, 1977)
	EC50-root	>200	(Mahmoud and Grime, 1977)

[a] Values for phytotoxicity thresholds (PT's) are mg kg^{-1} plant tissue; values for effective concentrations (ECs) are mg l^{-1}

what might be observed in the field due to the assumptions implicit in the experimental design.

5. Conclusions

Based on EC50-plant values, it appears that slender wheatgrass and common wheat are sensitive to Mn relative to the other restoration grass species. From our data it appears that redtop, tufted hairgrass, Basin wildrye and big bluegrass would all be good species for restoration of Mn contaminated sites. The thresholds provided here should be more useful for risk assessors than the currently available and widely used thresholds determined for crop plants using similar methodology. Our observation that the agronomic species used in this experiment (common wheat) was the least Mn tolerant species is important because it indicates that risk assessments conducted using thresholds for agronomic species may call for remediation efforts that might not be justifiable where restoration grass species are to be used.

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References

Alloway, B.J., 1990. Soil processes and the behavior of metals. In: Alloway, B.J. (Ed.), Heavy Metals in Soils. John Wiley & Sons, New York, pp. 7–28.

Bradley, R., Burt, A.J., Read, D.J., 1982. The biology of mycorrhiza in the Ericaceae. VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytologist 91, 197–209.

Brown, M.T., Wilkins, D.A., 1985. Zinc tolerance in mycorrhizal Betula. New Phytologist 99, 101–106.

Calabrese, E.J., Baldwin, L.A., 2003. Toxicology rethinks its central belief – hormesis demands a reappraisal of the way risks are assessed. Nature 421, 691–692.

Cenni, E., Bussotti, F., Galeotti, L., 1998. The decline of a *Pinus nigra* Arn. reforestation stand on a limestone substrate: the role of nutritional factors examined by means of foliar diagnosis. Annales Des Sciences Forestieres 55, 567–576.

Choi, J., Pak, C., Lee, C.W., Choi, J.M., Pak, C.H., 1996. Micronutrient toxicity in French marigold. Journal of Plant Nutrition 19, 901–916.

De Marco, D., Li, C., Randall, P., 1995. Manganese toxicity in *Trifolium balansae*. *T. resupinatum*, *T. subterraneum*, *Medicago murex*, *M. polymorpha*, *M. sativa*, *Lotus pedunculatus*, and *Ornithopus compressus*: relative tolerance and critical toxicity concentrations. Australian Journal of Experimental Agriculture 35, 367–374.

de Varennes, A., Carneiro, J.P., Goss, M.J., 2001. Characterization of manganese toxicity in two species of annual medics. Journal of Plant Nutrition 24, 1947–1955.

Ebbs, S., Kochian, L., 1997. Toxicity of zinc and copper to Brassica species: implications for phytoremediation. Journal of Environmental Quality 26, 776–781.

Ehinger, L.H., Parker, G.R., 1979. Tolerance of *Andropogon scoparius* to copper and zinc. New Phytologist 83, 175–180.

Fageria, N.K., 2001. Adequate and toxic levels of copper and manganese in upland rice, common bean, corn, soybean, and wheat grown on an Oxisol. Communications in Soil Science and Plant Analysis 32, 1659–1676.

Foy, C.D., Chaney, R.L., White, M.C., 1978. The physiology of metal toxicity in plants. Annual Review of Plant Physiology 29, 511–566.

Foy, C.D., Fleming, A.L., Schwartz, J.W., 1973. Opposite aluminum and manganese tolerances of two wheat varieties. Agronomy Journal 65, 123–126.

Ghosh, R., Banerjee, D.K., 1997. Complexation of trace metals with humic acids from soil, sediment and sewage. Chemical Speciation and Bioavailability 9, 15–19.

Gough, L.P., Shacklette, H.T., Case, A.A., 1979. Elemental concentrations toxic to plants, animals and man, Geological Survey Bulletin no. 1466. US Department of Interior Geological Survey, Washington, DC.

Hogan, G.D., Rauser, W.E., 1979. Tolerance and toxicity of cobalt, copper, nickel and zinc in clones of *Agrostis gigantea*. New Phytologist 83, 665–670.

Horst, W.J., 1988. The physiology of manganese toxicity. In: Graham, R.D., Hannam, R.J., Uren, N.C. (Eds.), Manganese in Soils and Plants. Kluwer, Dordrecht, pp. 175–188.

Humphreys, M.O., Nicholls, M.K., 1984. Relationships between tolerance to heavy metals in *Agrostis capillaris* L. (*A. tenuis* Sibth.). New Phytologist 98, 177–190.

Jackson, M.B., Lee, C.W., Schumacher, M.A., Duysen, M.E., Self, J.R., Smith, R.C., 1995. Micronutrient toxicity in buffalograss. Journal of Plant Nutrition 18, 1337–1349.

Jones, M.D., Hutchinson, T.C., 1986. The effect of mycorrhizal infection on the response of *Betula papyrifera* to nickel and copper. New Phytologist 102, 429–442.

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants. CRC Press, Boca Raton, FL.

Kroeze, C., Pegtel, D.M., Blom, C.J.C., 1989. An experimental comparison of aluminum and manganese susceptibility in *Antennaria dioica*, *Arnica montana*, *Viola canina*, *Filago minima* and *Deschampsia flexuosa*. Acta Botanica Neerlandica 38, 165–172.

Langheinrich, U., Tischner, R., Godbold, D.L., 1992. Influence of a high Mn supply on Norway spruce (*Picea abies* (L.) Karst.) seedlings in relation to the nitrogen source. Tree Physiology 10, 259–271.

Lee, C.W., Jackson, M.B., Duysen, M.E., Freeman, T.P., Self, J.R., 1996. Induced micronutrient toxicity in 'Touchdown' Kentucky bluegrass. Crop Science 36, 705–712.

Lidon, F.C., 2002. Rice plant structural changes by addition of excess manganese. Journal of Plant Nutrition 25, 287–296.

Macnicol, R.D., Beckett, P.H.T., 1985. Critical tissue concentrations of potentially toxic elements. Plant and Soil 85, 107–129.

Mahmoud, A., Grime, J.P., 1977. Comparison of susceptibility of *Arrhenatherum elatius* (L.) Beauv, Ex. Presl. Jc., *Agrostis tenuis* Sibth., *Deschampsia flexuosa* and *Festuca ovina* L. to manganese toxicity. Plant and Soil 47, 559–565.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Martino, E., Turnau, K., Girlanda, M., Bonfante, P., Perotto, S., 2000. Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of zinc ions. Mycological Research 104, 338–344.

Miles, L.J., Parker, G.R., 1979. The effect of soil-added cadmium on several plant species. Journal of Environmental Quality 8, 229–232.

Ohki, K., 1984. Manganese deficiency and toxicity effects on growth, development, and nutrient composition in wheat. Agronomy Journal 76, 213–218.

Ohki, K., 1985. Manganese deficiency and toxicity effects on photosynthesis, chlorophyll, and transpiration in wheat. Crop Science 25, 187–191.

Paschke, M.W., Redente, E.F., 2002. Copper toxicity thresholds for important restoration grass species of the western United States. Environmental Toxicology and Chemistry 21, 2692–2697.

Paschke, M.W., Redente, E.F., Levy, D.B., 2000. Zinc toxicity thresholds for important reclamation grass species of the western United States. Environmental Toxicology and Chemistry 19, 2751–2756.

Pedersen, M.B., Kjaer, C., Elmegaard, N., 2000. Toxicity and bioaccumulation of copper to black bindweed (*Fallopia convolvulus*) in relation to bioavailability and the age of soil contamination. Archives of Environmental Contamination and Toxicology 39, 431–439.

Pollard, A.J., 1980. Diversity of metal tolerances in *Plantago lanceolata* L. from the southeastern United States. New Phytologist 86, 109–117.

Prodgers, R.A., Inskeep, W.P., 1991. Heavy metal tolerance of inland saltgrass (*Distichlis spicata*). Great Basin Naturalist 51, 271–278.

Quartin, V.M.L., Antunes, M.L., Muralha, M.C., Sousa, M.M., Nunes, M.A., 2001. Mineral imbalance due to manganese excess in triticales. Journal of Plant Nutrition 24, 175–189.

Ramachandran, V., D'Souza, T.J., 1997. Extractable zinc and manganese as related to applied cadmium in contrasting Indian soils. Chemical Speciation and Bioavailability 9, 131–144.

Reisenaur, H.M., 1988. Determination of plant-available soil manganese. In: Graham, H.M., Hannam, R.J., Uren, N.C. (Eds.), Determination of plant-available soil manganese. Kluwer, Dordrecht, pp. 87–100.

Ross, S.M., Kaye, K.J., 1994. The meaning of metal toxicity in soil-plant systems. In: Ross, S.M. (Ed.), The meaning of metal toxicity in soil-plant systems. John Wiley & Sons, Chichester, pp. 27–61.

Scott, B.J., Fisher, J.A., Spohr, L.J., 1998. Manganese tolerance in a range of wheat genotypes. Communications in Soil Science and Plant Analysis 29, 219–235.

Smith, K.A., Paterson, J.E., 1990. Manganese and cobalt. In: Alloway, B.J. (Ed.), Manganese and cobalt. John Wiley & Sons, New York, pp. 224–244.

Stevenson, F.J., Ardakani, M.S., 1972. Organic matter reactions involving micronutrients in soils. In: Mortveldt, J.J., Giordano, P.M., Lindsay, W.L. (Eds.), Organic matter reactions involving micronutrients in soils. Soil Science Society of America, Madison, pp. 79–114.

Symeonidis, L., McNeilly, T., Bradshaw, A.D., 1985. Differential tolerance of three cultivars of *Agrostis capillaris* L. to cadmium, copper, lead, nickel and zinc. New Phytologist 101, 309–315.

Van Tichelen, K.K., Colpaert, J.V., Vangronsveld, J., 2001. Ectomycorrhizal protection of *Pinus sylvestris* against copper toxicity. New Phytologist 150, 203–213.

Wong, M.H., Lau, W.M., Li, S.W., Tang, C.K., 1983. Root growth of two grass species on iron ore tailings at elevated levels of manganese, iron, and copper. Environmental Research 30, 26–33.

Wu, L., Kruckeberg, A.L., 1985. Copper tolerance in two legume species from a copper mine habitat. New Phytologist 99, 565–570.

Zheljazkov, V.D., Nielsen, N.E., 1996. Effect of heavy metals on peppermint and cornmint. Plant and Soil 178, 59–66.